

Systematic Significance of Pollen Morphology in *Clausena* (Rutaceae) from China

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(Accepted on May 28, 2012)

Clausena is the most widely distributed genus in the subfamily *Aurantioideae* of the family *Rutaceae*. Morphological features of pollen grains from 10 species and two varieties of *Clausena* in China were examined using scanning electron microscopy (SEM). The pollen grains are small to medium in size and subprolate in shape, with a few being prolate. The apertures are usually 3-colporate, except one species (*C. lansium*) being 3- or 4-colporate. The morphology of the pollen grains shows significant variation in the exine ornamentation, viz. striato-reticulate, striate, rugulo-reticulate or striato-perforate. Five species and two varieties, viz. *C. anisum-olens*, *C. dunniana* var. *dunniana*, *C. excavata* var. *excavata*, *C. inolida*, *C. lansium*, *C. odorata* and *C. vestita* commonly show the exine ornamentation of striato-reticulate type, but the width of murus and lumen vary between species. *C. lenis* and *C. yunnanensis* have the rugulo-reticulate exine ornamentation while *C. excavata* var. *quadrangulata* and *C. dunniana* var. *robusta* with *C. emarginata* are characterized with striate and striato-perforate exine ornamentation respectively. *Clausena* is euryhalophilous and the pollen characteristics appear to be informative and useful for distinguishing species and for elucidating the relationships among some species.

Key words: *Clausena*, pollen morphology, *Rutaceae*, systematic significance.

The genus *Clausena* Burm. f. was first described in 1768 (Burman 1768). Species of *Clausena* can be found from North-Western India to China, Northern Australia and New Guinea. Moreover, there is a group of three species that covers a wide range in Africa. Based on the morphology of *Clausena*, Swingle and Reece (1967) proposed 23 species and 12 varieties while Molino (1994) recognized 15 species and six varieties. In the recent revision, Kubitzki et al. (2011) mentioned 15 species. There are 10 species and two varieties occurring in China according

to the Flora of China (Huang 1997, Zhang et al. 2008), along with a new species and a variety (Yu and Wong 1992). In this moderately large and the most widely distributed genus in the subfamily *Aurantioideae*, many species are found to be of value for their edible fruits (as Wampee), for use as rootstocks, for their essential oils, or for planting as ornamentals because of their abundant white flowers and handsome foliage; many species of *Clausena* are locally used as important medicine.

According to Swingle and Reece (1967),

Table 1. Voucher information for taxa of *Clausena* used in this study

Taxon	Locality	Voucher specimen	Figure
<i>C. anisum-olens</i> (Blanco) Merr. (1)	Longzhou, Guangxi	Z. J. Yu 008 B F (CANT)	Fig. 1 A-C
<i>C. anisum-olens</i> (Blanco) Merr. (2)	Longzhou, Guangxi	F. J. Mou 206 (IBSC)	Fig. 1 D-F
<i>C. dunniana</i> H. Lév. var. <i>dunniana</i> (1)	Yangshan, Guangdong	F. J. Mou 144 (IBSC)	Fig. 1 G-I
<i>C. dunniana</i> var. <i>dunniana</i> (2)	Yongfu, Guangxi	Tam Ying-wah 99 (SYS)	Fig. 1 J-L
<i>C. dunniana</i> H. Lév. var. <i>robusta</i> (Tanaka) Huang	Xingyi, Guizhou	Guizhou Exp. 6629 (IBK)	Fig. 2 A-C
<i>C. emarginata</i> Huang	Funing, Yunnan	J. X. Ye & Q. Li 225 (SYS)	Fig. 2 D-F
<i>C. excavata</i> Burm. f. var. <i>excavata</i> (1)	Wanning, Hainan	F. J. Mou 166 (IBSC)	Fig. 2 G-I
<i>C. excavata</i> var. <i>excavata</i> (2)	Jinping, Yunnan	Sino-Russian Exp. 25 (IBSC)	Fig. 2 J-L
<i>C. excavata</i> Burm. f. var. <i>quadrangulata</i> Z. J. Yu & C. Y. Wong (1)	Pingxiang, Guangxi	Z. J. Yu 0021A (CANT)	Fig. 3 A-C
<i>C. excavata</i> var. <i>quadrangulata</i> (2)	Longzhou, Guangxi	F. J. Mou 208 (IBSC)	Fig. 3 D-F
<i>C. inolida</i> Z. J. Yu & C. Y. Wong	Pingxiang, Guangxi	Z. J. Yu 0013 A G (CANT)	Fig. 3 G-I
<i>C. lansium</i> (Lour.) Skeels (1)	Guangzhou, Guangdong	F. J. Mou 102 (IBSC)	Fig. 3 J-L
<i>C. lansium</i> (Lour.) Skeels (2)	Xishuangbanna Tropical Botanical Garden, Yunnan	F. J. Mou 45 (IBSC)	Fig. 4 A-C
<i>C. lenis</i> Drake	Mengla, Yunnan	J. H. Zhang 13626 (IBSC)	Fig. 4 D-F
<i>C. odorata</i> Huang (1)	Mojiang, Yunnan	H. T. Tsai 55862 (KUN)	Fig. 4 G-I
<i>C. odorata</i> Huang (2)	Yongde, Yunnan	E. D. Liu 134 (KUN)	Fig. 4 J-L
<i>C. vestita</i> Tao	Puge, Sichuan	Nanshuibeidiao 7414 (KUN)	
<i>C. yunnanensis</i> Huang	Napo, Guangxi	South China Exp. 1096 (IBSC)	Fig. 5 D-F

Clausena is classified into the subtribe *Clauseninae* of the tribe *Clauseneae* in the subfamily *Aurantioideae* of *Rutaceae*, along with the genera *Glycosmis* and *Murraya* based on morphological characters and the subtribe *Clauseninae* comprises the tribe *Clauseneae* with *Merrilliinae* (only *Merrillia*) and *Micromelinae* (only *Micromelum*). Species of *Clausena* are more closed to those of *Murraya* Sect. *Bergera* based on morphology and phytochemistry (Kong et al. 1986, But et al. 1986, But et al. 1988, Li et al. 1988, Samuel et al. 2001), palynology (Mou and Zhang 2009), chromosome (Guerra et al. 2000) and molecular phylogeny (Samuel et al. 2001, Pfeil and Crisp 2008, Bayer et al. 2009, But et al. 2009, Morton 2009). In the newest family treatment of *Rutaceae*, Kubitzki et al. (2011) defined the *Bergera* Alliance that includes *Clausena*, *Bergera*, *Micromelum* and *Glycosmis*.

Molino (1994) divided *Clausena* into

four sections, viz., *Piptostylis*, *Drakanthus*, *Clausena* and *Axillanthus*. However, determining infrageneric relationships in *Clausena* is extremely difficult because the delimitation of some species, such as *C. anisata* and *C. dunniana*, is obscure and the majority of the characters used to determine species are quantitative features of leaves and flowers, including gynophore, ovary and style. Many species of this genus are still imperfectly studied with respect to the minute flower characters. Also, no detailed molecular phylogenetic study of *Clausena* has been published until now. The important characters that may help to define the natural groups of species are found in the pistil (Tillson and Bamford 1938). The most distinctive morphological characters of *Clausena* are the gynophore, which in the typical species is a large, well-developed, hourglass-shaped structure supporting the ovary. The current classifications of *Clausena* are mainly based on

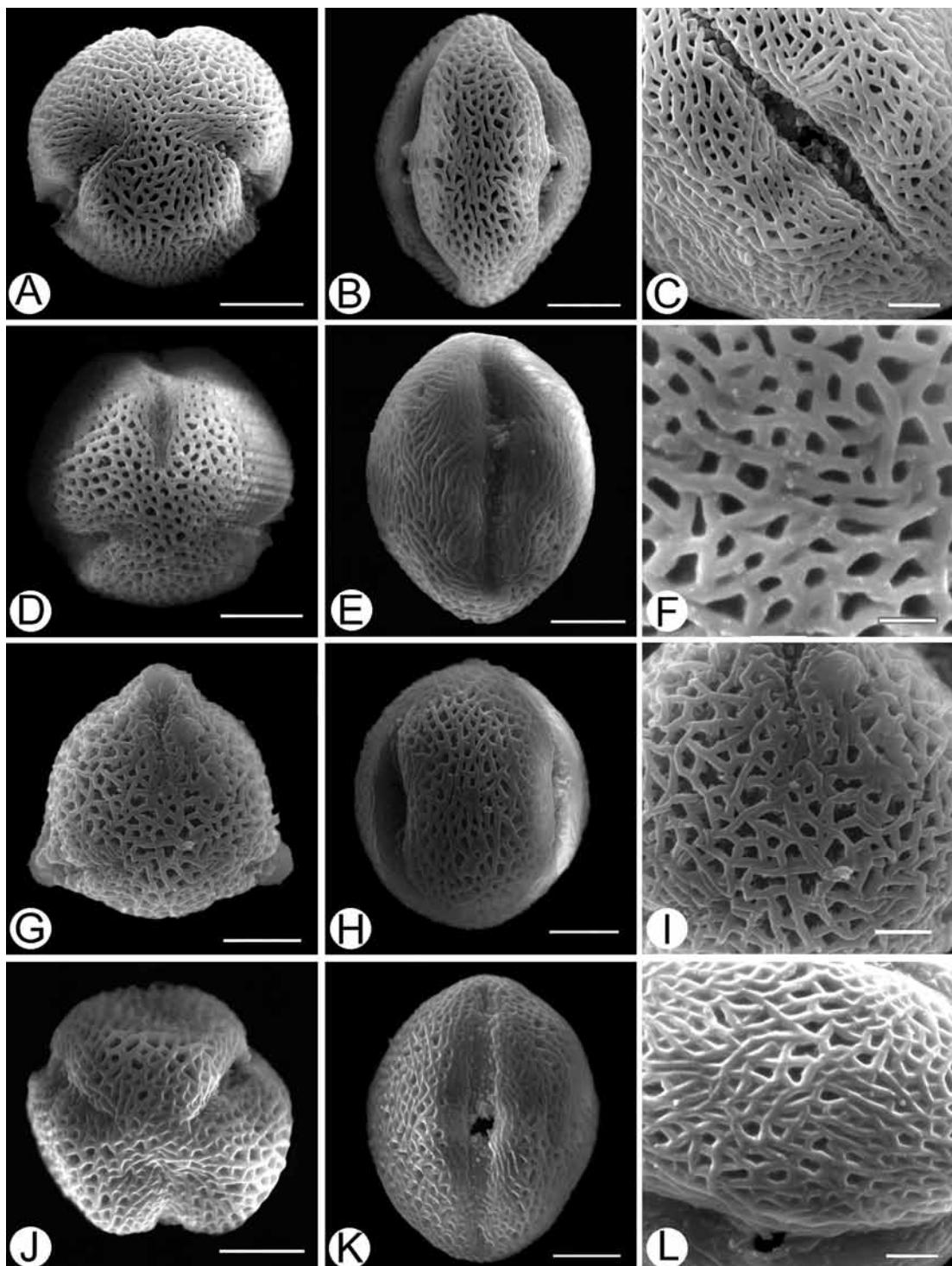


Fig. 1. Pollen morphology of *Clausena*. A–C. *C. anisum-oleans* (1). D–F. *C. anisum-olen* (2). G–I. *C. dunniana* var. *dunniana* (1). J–L. *C. dunniana* var. *dunniana* (2). Scale bar: A, B, D, E, G, H, J, K = 5 μm ; C, I, L = 2 μm ; F = 1 μm .

morphology (Swingle and Reece 1967, Molino 1994).

Pollen morphology has provided very useful taxonomic evidence in *Rutaceae* (Liu 1987, Grant et al. 2000, Mou and Zhang 2009). Liu (1987) reported that pollen grains of *Rutaceae* were colporate and four species of *Clausena* belonged to the 3-colporate with reticulate exine ornamentation type. Grant et al. (2000) recognized five pollen types in the subfamily *Aurantioideae*, based mainly on aperture number and exine ornamentation and the pollen grains show a high degree of intergeneric variation. All genera of the tribe *Clauseneae* had 3-colporate pollen grains with striate or striato-reticulate exine ornamentation. Yu et al. (1992) divided the pollen of *Clausena* into two types according to the exine ornamentation, namely striato-reticulate type (including *Clausena emarginata*, *C. excavata* var. *quadrangulata*) and reticulate type (including *C. anisum-olens*, *C. inolida* and *C. lansium*). In view of rugulate to foveolate exine in *Murraya* sect. *Bergera* and cross-striate in *Murraya* sect. *Murraya* being, Mou and Zhang (2009) treated sect. *Bergera* as a separate genus and suggested that *Murraya* s.s. was closer to *Micromelum* and *Merrillia*.

The present study aims to examine the variation in pollen morphology of the genus, and provides additional supports to its systematic implications in relation to the existing classification, especially classification treatments of difficult species or section division currently recognized within *Clausena*. Congruence between pollen types and the currently accepted classification is discussed, as well as the systematic implications of pollen morphology for the genus.

Materials and Methods

Pollen materials of 18 samples from 10 species and two varieties of *Clausena* were studied, and all samples were from China. Pollen grains were obtained from specimens in the herbaria of the South China Botanical Garden (IBSC), Kunming Institute of Botany (KUN), Guangxi Institute of

Botany (IBK), Sun Yat-Sen University (SYS), and the South China Agricultural University (CANT), as well as from living samples collected in Botanic Gardens or in the field. All voucher specimens for pollen samples are listed in Table 1.

All samples were acetolysed for 10 min at 95–100°C (Erdtman 1960, 1969). After acetolysis, the samples were treated in 70 % alcohol three times for 10 min with ultrasound and stored in 70% ethanol. For examination with SEM, pollen samples were air-dried from 70% ethanol onto specimen stubs covered with negative photographic film, sputter-coated with gold-palladium and observed using a JSE-6360LV SEM at a voltage of 10 KV. All measurements were based on at least 20 pollen grains and the terminology follows Grant et al. (2000), Erdtman (1969) and Wei (2003).

Results

The morphology of pollen grains of *Clausena* is summarized in Table 2 and the SEM images are shown in Figures 1 to 5. The pollen grains of all species examined in this study are generally isopolar, monads and colporate, and are small in size; the smallest grains (*C. yunnanensis*) were P = 18.29 μm and E = 13.34 μm in size while the largest ones (*C. lenis*) P = 27.07 μm and E = 21.84 μm. The range of P/E varies from 1.11 to 1.58. The pollen grains of most species are subprolate in shape while a few prolate. Aperture number is 3, except for *C. lansium* which had 3 or 4 (Table 2; Fig. 3, J–K, Fig. 4, A–C). Pollen of *Clausena* can be divided into four types mainly based on the features of exine ornamentation, which are striato-reticulate, striate, rugulo-reticulate and striato-perforate type.

Striato-reticulate type: seven taxa in this genus share the same type of exine ornamentation, which are *C. anisum-olens* (Fig. 1, A–F), *C. dunniana* var. *dunniana* (Fig. 1, G–L), *C. excavata* var. *excavata* (Fig. 2, G–L), *C. inolida* (Fig. 3, G–I), *C. lansium* (Fig. 3, J–L, Fig. 4, A–C), *C. odorata* (Fig. 4, G–L) and *C. vestita* (Fig. 5, A–C). This type is characterized with reticulate

Table 2. Morphological characters of pollen grains in *Clausena*

Taxon	Aperture	Polar axis (μm)	Equatorial axis (μm)	Shape	P/E	Polar view	Exine ornamentation	Width of murus (μm)	Width of lumen (μm)	M/L
<i>C. anisum-o-lens</i> (1)	3-colporate	19.52±1.75 (17.7-22.4)	15.90±0.51 (14.7-17.9)	subprolate	1.23	circular, with the colpori sunken	finely striato-reticulata	0.339±0.0018	0.896±0.057	0.38
<i>C. anisum-o-lens</i> (2)	3-colporate	17.61±1.29 (15.3-19.6)	14.78±0.72 (13.0-16.1)	subprolate	1.19	circular, with the colpori sunken	finely striato-reticulata	0.289±0.001	0.564±0.019	0.51
<i>C. duminiana</i> var. <i>duminiana</i> (1)	3-colporate	20.43±1.99 (18.3-23.1)	16.71±0.65 (14.8-18.1)	subprolate	1.22	circular, with 3 lobes	finely striato-reticulata	0.336±0.0006	0.944±0.036	0.36
<i>C. duminiana</i> var. <i>duminiana</i> (2)	3-colporate	20.30±0.79 (18.9-22.0)	15.59±0.54 (14.6-17.7)	subprolate	1.30	circular, with the colpori sunken	finely striato-reticulata	0.360±0.0021	0.897±0.032	0.40
<i>C. duminiana</i> var. <i>robusta</i>	3-colporate	21.59±1.17 (19.2-23.9)	15.26±0.55 (14.4-16.7)	prolate	1.41	circular, with 3 lobes	coarsely striato-perforate	0.308±0.0012	1.103±0.087	0.28
<i>C. emarginata</i>	3-colporate	22.01±0.40 (20.8-22.9)	16.19±0.47 (15.0-17.7)	prolate	1.36	circular, with quite sunken colpori	finely striato-perforate	0.343±0.0007	0.924±0.024	0.37
<i>C. excavata</i> var. <i>excavata</i> (1)	3-colporate	20.19±0.76 (18.7-21.7)	15.70±0.34 (14.6-16.7)	subprolate	1.29	circular	finely striato-reticulata	0.305±0.001	0.847±0.029	0.36
<i>C. excavata</i> var. <i>excavata</i> (2)	3-colporate	20.29±0.65 (12.6-18.4)	12.86±0.41 (14.7-11.9)	prolate	1.58	circular, with colpori sunken	finely striato-reticulata	0.299±0.0004	0.404±0.020	0.74
<i>C. excavata</i> var. <i>quadrangulata</i> (1)	3-colporate	22.17±2.15 (25.0-19.7)	15.09±1.13 (12.9-16.6)	prolate	1.47	circular, with colpori sunken	striae	0.315±0.0007		
<i>C. excavata</i> var. <i>quadrangulata</i> (2)	3-colporate	18.16±0.77 (17.1-20.8)	14.89±0.30 (13.7-16.0)	subprolate	1.22	circular, with colpori slightly sunken	striae	0.401±0.0018		
<i>C. inolida</i>	3-colporate	20.40±1.60 (18.6-22.9)	15.30±1.03 (13.2-17.0)	subprolate	1.33	circular, with the colpori sunken	coarsely striato-reticulata	0.341±0.0012	1.010±0.047	0.33
<i>C. lansium</i> (1)	3- or 4-colporate	19.18±0.62 (17.6-20.4)	15.58±0.74 (14.3-17.0)	subprolate	1.23	circular, with colpori slightly sunken	finely striato-reticulata	0.338±0.0014	0.810±0.019	0.42
<i>C. lansium</i> (2)	3- or 4-colporate	21.69±0.64 (20.2-23.4)	18.82±0.66 (17.3-20.5)	subprolate	1.15	circular, with colpori slightly sunken	finely striato-reticulata	0.404±0.0014	0.733±0.024	0.55
<i>C. lens</i>	3-colporate	27.07±1.02 (25.5-29.2)	21.84±0.60 (20.6-23.3)	subprolate	1.24	circular, with quite sunken colpori	finely rugulo-reticulate with microperforation	0.346±0.0025		
<i>C. odorata</i> (1)	3-colporate	20.32±1.55 (17.4-22.1)	17.00±0.73 (15.6-18.4)	subprolate	1.20	circular, with colpori slightly sunken	finely striato-reticulata	0.411±0.0024	0.997±0.061	0.41
<i>C. odorata</i> (2)	3-colporate	18.92±1.90 (16.9-22.2)	17.02±1.22 (14.8-19.2)	subprolate	1.11	circular, with colpori slightly sunken	finely striato-reticulata	0.380±0.0014	0.465±0.0001	0.82
<i>C. vestita</i>	3-colporate	24.52±1.26 (22.3-26.7)	17.00±0.85 (15.6-19.0)	prolate	1.44	circular, with the colpori sunken	finely striato-reticulata	0.325±0.0014	0.780±0.034	0.42
<i>C. yunnanensis</i>	3-colporate	18.29±0.89 (16.4-19.9)	13.34±0.24 (12.5-14.1)	prolate	1.37	circular, with colpori slightly sunken	rugulo-reticulate with microperforation	0.267±0.0006	0.379±0.005	0.70

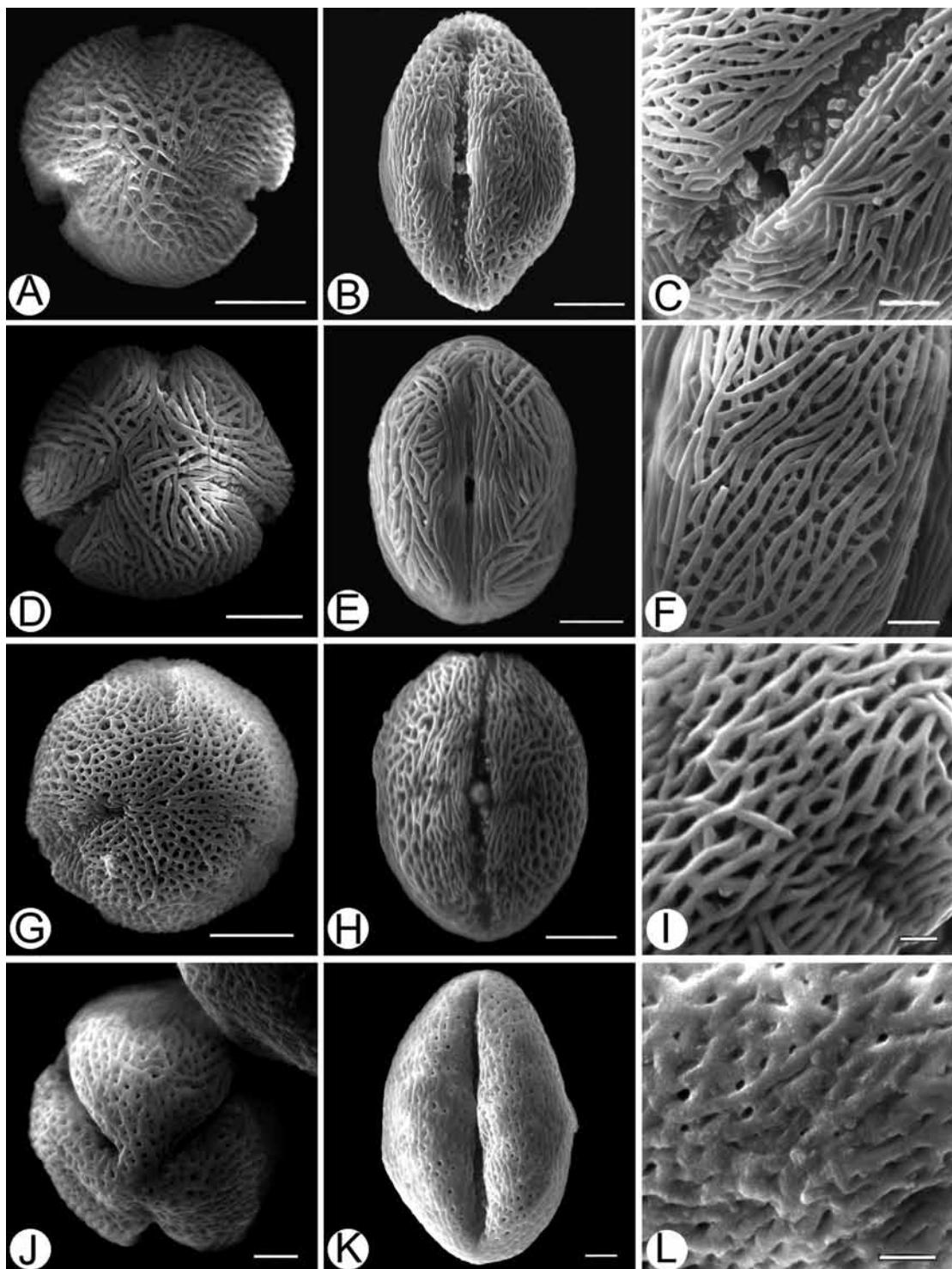


Fig. 2. Pollen morphology of *Clausena*. A–C. *C. dunniana* var. *robusta*. D–F. *C. emarginata*. G–I. *C. excavata* var. *excavata* (1). J–L. *C. excavata* var. *excavata* (2). Scale bar: A, B, D, E, G, H = 5 μm ; C, F, J, K = 2 μm ; I, L = 1 μm .

ornamentation, the width of the murus ranges from 0.289 to 0.411 μm , the width of the lumen from 0.404 to 1.010 μm and the ratio of width of murus and lumen from 0.36 to 0.82 (Table 2).

Striate type: only one variety, *C. excavata* var. *quadrangulata* (Fig. 3 A–F) shows this type of ornamentation, and the width of the murus is from 0.315 to 0.401 μm (Table 2).

Rugulo-reticulate type: the striation is regulate with some microperforation. *Clausena lenis* (Fig. 4, D–F) and *C. yunnanensis* (Fig. 5, D–F) are included into this type, and the width of the murus ranges from 0.267 to 0.346 μm (Table 2).

Striato-perforate type: the exine ornamentation is striate and distributed with microperforation. *Clausena dunniana* var. *robusta* (Fig. 2, A–C), *C. emarginata* (Fig. 2, D–F) exhibit this type and the width of the murus ranges from 0.308 to 0.343 μm (Table 2).

Discussion

In the present study, the pollen morphology of three species and one variety in *Clausena* was reported for the first time, namely *C. dunniana* var. *robusta*, *C. odorata*, *C. vestita* and *C. yunnanensis*. The exine ornamentation of the seven taxa is striato-reticulate and not reticulate, as described by Liu (1987). Liu (1987) reported that *C. excavata*, *C. lenis*, *C. dunniana* and *C. lansium* were characterized with 3-colporate pollen grains with thin-reticulate exine ornamentation and Yu et al. (1992) described ornamentation of *C. anisum-olens*, *C. inolida* and *C. lansium* as reticulate. Grant et al. (2000) reported subprolate, 3-colporate and microstriato-reticulate pollen grains for *C. inequalis*, and described the species as the *Clausena inequalis* type with *Glycosmis pentaphylla* and *Feroniella pubescens*. The rugulo-reticulate type was observed in *C. lenis* and *C. yunnanensis*, the striate type for *C. excavata* var. *quadrangulata* and the striato-perforate type for *C. dunniana* var. *robusta* and *C. emarginata* for the first time. The pollen morphology of *Clausena* is not accordant with the four sections of Molino (1994). In the revision

of Molino (1994), both of *C. anisum-olens* and *C. emarginata* were treated as synonyms of *C. sanki*, nevertheless, exine ornamentation of two species is striato-reticulate and striate-perforate, respectively.

Clausena lansium is a highly esteemed fruit tree (Wampee) and sour, subacid and sweet varieties are known. The pollen grains of this species are 3- or 4-colporate (Table 2; Fig. 3, J–L, Fig. 4, A–C), which is very different from other species which are exclusively 3-colporate. This species stands apart from all the other species of the genus in having a star-shaped bud (due to its five strongly carinate petals) and a 5-angled ovary, corresponding in position to the five petal keels in a cross section of the bud. The pistil is unusually large; the ovary is very strongly hirsute and merges into the style, which is slightly contracted at the base. Morphology of characters and pollen all supports its special position in *Clausena*.

Clausena excavata has the widest distribution in all *Clausena* species, ranging from Eastern Indian to southeastern Asia and many varieties have been described for the species (Molino 1994). The pollen morphology is variable in different varieties or from different locations, showing the finely striato-reticulate exine ornamentation in var. *excavata* and striate in var. *quadrangulata*. *Clausena excavata* has a rather full, smooth and hourglass-shaped gynophore supporting the more or less strongly hirsute ovary. The ovary tapers gradually into the thick style, which shows no constriction at its base as do the styles of many other species. The transaction of ovary is circular in *C. excavata* var. *excavata* while quadrangular in var. *quadrangulata*. The pollen morphology of *C. excavata* is congruent with the variable morphology of its many varieties.

Clausena dunniana is a widely-distributed species in China. This species is characterized by axillary flower clusters, non-terminal panicles (as in most other species) and the long pedicels of the flowers, which are unusual in *Clausena*. Moreover, most vegetative organs are variable in this species. However, the sunken colpori and

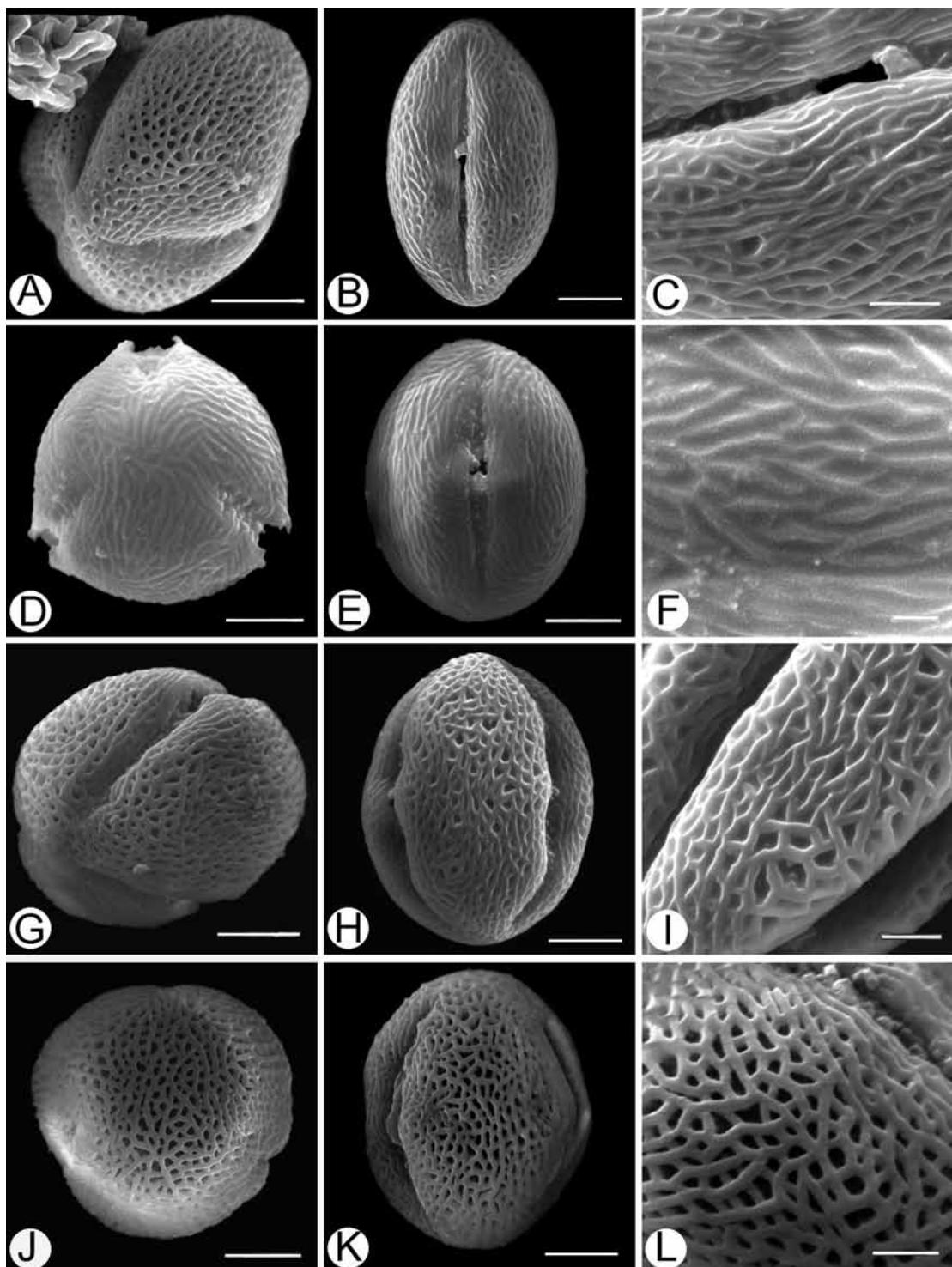


Fig. 3. Pollen morphology of *Clausena*. A–C. *C. excavata* var. *quadrangulata* (1). D–F. *C. excavata* var. *quadrangulata* (2). G–I. *C. inolida*. J–L. *C. lansium*. Scale bar: A, B, D, E, G, H, J, K = 5 μm ; C, I, L = 2 μm ; F = 1 μm .

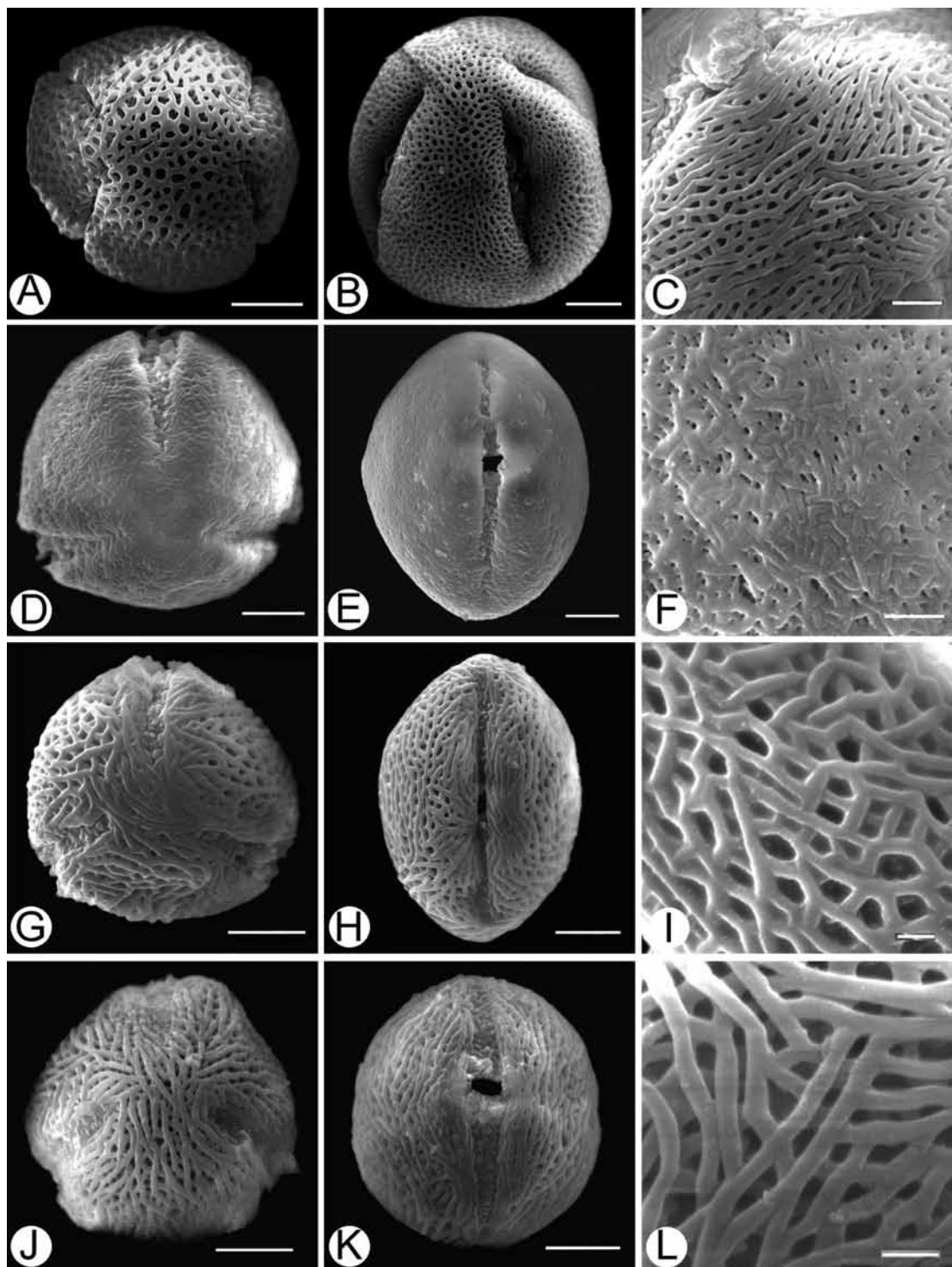


Fig. 4. Pollen morphology of *Clausena*. A–C. *C. lansium*. D–F. *C. lenis*. G–I. *C. odorata*. J–L. *C. odorata*. Scale bar: A, B, D, E, G, H, J, K = 5 μm ; C, F = 2 μm ; I, L = 1 μm .

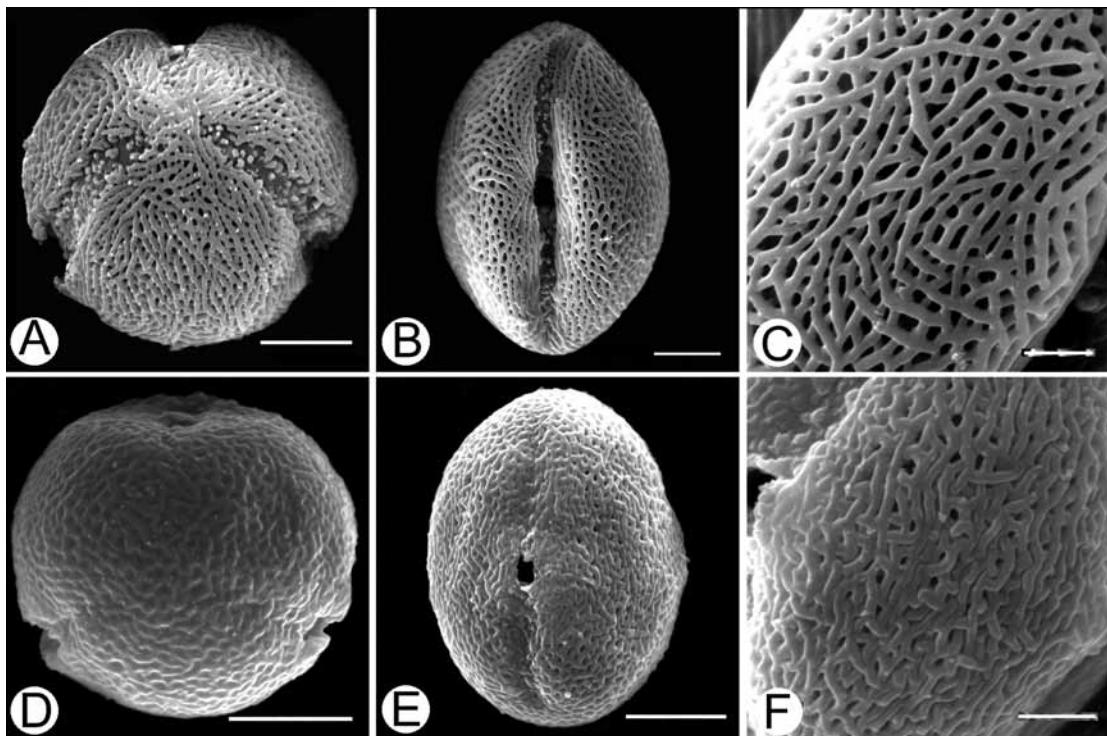


Fig. 5. Pollen morphology of *Clausena*. A–C. *C. vestita*. D–F. *C. yunnanensis*. Scale bar: A, B, D, E = 5 μm ; C, F = 2 μm .

finely striato-reticulate exine ornamentation are shown in var. *dunniana* while with three lobes and coarsely striato-perforate in var. *robusta*. Considering that the morphology of pollen is obviously variable between both varieties, the palynological characters, such as colpori and exine ornamentation, can be used to identify this species and variety.

Clausena inolida was first described by Yu and Wong (1992), based on a few specimens collected from the same location in Guangxi Province. Considering the very high similarity of the pollen morphology between *C. inolida* and *C. dunniana*, as well as other characters (leaf and flower), *C. inolida* should be considered a synonym of *C. dunniana*.

Clausena lenis is remarkable and different from other species of the genus *Clausena* in having leathery petals and a most peculiar gynophore, which is not hourglass-shaped as in almost all the other species. The style is slender,

two to three times as long as the ovary, by far the longest of any species in the genus, and contracted below, where it joins the ovary. According to the revision of Molino (1994), *C. lenis* is the only species in *Clausena* sect. *Drakanthus*. The striate-perforate pollen type of *C. lenis* certainly puts it into an isolated position, probably with affinities to *Bergera* (Mou and Zhang 2009). Although the exine ornamentation of *C. lenis* is similar to that of *C. yunnanensis*, both are different in other characters, such as leaf, petal, stamen and pistil.

In *Clausena*, pollen morphology, such as exine ornamentation is consistent with its other characters and shows a very important taxonomic implication in disposing of some systematic problems.

The authors are grateful to the curators and directors of the herbaria CANT, IBK, IBSC, KUN and SYS for providing materials for this study. We are indebted to Ms. Xiao-Ying Hu from South

China Botanical Garden, CAS, for technical assistance with SEM observation. This project was supported by National Science Foundation of China (30970182, 30870367, 30570314) and the Knowledge Innovation Program of the Chinese Academy of Sciences, grant No. KSCX2-YW-Z-027.

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牟鳳娟, 張奠湘: 中国産 *Clausena* 属 (ミカン科)
における花粉形態の分類学的意義

ミカン科ミカン亜科の *Clausena* 属の中国産 10 種 1 変種の花粉形態を調べた。花粉粒は小型から中型で、形状は亜長球型 subprolate。発芽溝は、内口式 3 溝型 3-colporate あるいは内口式 4 溝型 4-colporate である。*C. lansium* を除いて、普通内口式 3 溝型であった。外被（外壁）の模様は大きな変異を示した、すなわち、類線状網状紋型 striato-reticulate, 線状紋型 striate, しわ状網状紋型 rugulo-reticulate, あるいは類線状微散孔型 striato-perforate であった。*C. anisumolens*, *C. inolida*, *C. lansium*, *C. odorata*, *C. vestita*, *C. dunniana* var. *dunniana*, *C. excavata* var. *excavata*

の 5 種 2 変種は共通して類線状網目型であったが、*凹 murus* と網目 *lumen* の幅は種によって異なっていた。*C. lenis* と *C. yunnanensis* の 2 種はしわ状網目型であった。一方、*C. emarginata*, *C. excavata* var. *quadrangulata*, *C. dunniana* var. *robusta* は線状紋型及び類線状微散孔型であった。*Clausena* 属は多類型的であり、花粉形態に関する特徴は種の識別やいくつかの種間関係を説明するために有益かつ有効であると考えられる。

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